Laboratory Safety
Bio 181

I. General practices:

1. First and foremost, to be safe in any lab, plan your experiments well, do not hurry when working and leave time to clean up properly.
2. **Wear a lab coat when working with microorganisms or toxic chemicals (e.g., ethidium bromide)**

II. Specific practices:

1. Eating and drinking are not permitted in the lab.
2. Wash your hands before beginning and after finishing work in the laboratory.
3. Long hair should be tied back when working in the laboratory. Wear closed toe shoes whenever possible to protect your feet.
4. Clean up as you proceed through experiments and keep your work area organized.
5. Disinfect your lab bench at the beginning and end of each lab.
6. Wear gloves and protective goggles or eyewear when working with toxic chemicals or UV light.
7. Minimize clutter on your lab bench. Put away extra notebooks, books, coats, etc. so they will not come in contact with your work. Be sure that backpacks are stored safely out of the way.
8. Never mouth pipet.
9. Clearly label **all** tubes that are in use.
10. Keep hot burners and sharp objects towards the center of the bench to prevent accidental contact.
11. Please report frayed cords or loose electrical connections to your instructor.
12. Dispose of any tubes, plates, pipet tips, etc. containing microorganisms in the biohazard autoclave bags for sterilization. (These bags are not for materials contaminated with chemicals—they are for microorganisms only.)
13. Dispose of broken glass and "sharps" in the containers clearly designated for these.
14. All ethidium bromide usage is to occur in the single designated area in the adjacent laboratory.
15. Be sure you know where the following safety equipment is in the lab and how to use it:

    - Eyewash
    - Shower
    - Fire blanket
    - Fire extinguisher
    - First aid kit
    - Nearest door to exit the lab
    - Fire exit from the building
    - Nearest phones to dial for help are in the stockroom, department office and the central lobby of Sequoia hall by the elevators.
    - **Dial 911 from any of these phones.**

16. Immediately report any accident—no matter how small—to your instructor.

III. Biohazards

1. Biohazards are organisms, viruses or other biological materials that are potentially hazardous. The other category could be defined to include blood products, plasmids and other forms of DNA or RNA, prions,

enzymes and proteins and everything else produced by the biological world. Since most, but necessarily all, of these are destroyed by autoclaving the standard way to handle biohazardous waste is to collect it in an autoclavable bag and, once full, the bag is autoclaved to kill all organisms and destroy viruses. Following this the bag may be disposed of safely.

2. The primary biohazard we will use in this course is *E. coli.* However, we will use strains derived from the strain K-12. This strain of *E. coli* is thought to be entirely safe for routine lab use since it is unable to survive outside the lab, unable to transfer plasmid DNA to other bacteria and unable to infect humans. We will, however, treat everything contaminated with live bacteria as biohazard and dispose of all of these materials in biohazard bags, **with the exception of all contaminated sharps or glass,** which must be collected in separate sharps or glass containers designated for biohazards. We also will disinfect the lab bench at the beginning and end of each lab.

IV. Physical Hazards

1. **Ultraviolet (UV) light:**

Ultraviolet (UV) light is a form of high energy radiation, with wavelengths just shorter and energies just higher than the visible spectrum. In this lab we will use UV illumination to visualize DNA stained with ethidium bromide (EtBr). EtBr is an intercalating agent that binds to DNA and fluoresces under UV light, allowing DNA to be visualized.

UV light damages DNA and kills cells. It is so effective in damaging DNA and cells that it is sometimes used to "surface sterilize" laboratory benchtops between times of use. UV light can cause **eye damage**, so do not look at it with unprotected eyes.

In this lab we will use UV light to illuminate our gels following staining with EtBr. For any unshielded transilluminators be sure to wear a UV protective face shield. The smaller transilluminator we will use routinely does not require the use of a face-mask as a magnetic safety switch prevents the UV light from being turned on unless the shield or a camera is in place.

2. **Electricity:**

Electrophoresis experiments present a potential shock hazard. The electrophoretic gel boxes used in this class are designed so that the electrodes must be disconnected from the power source before the box can be opened. However, since any surface can become conductive, it is advisable not to touch any part of the apparatus while the unit is on.

3. **Centrifugation**:

Benchtop microcentrifuges and large floor centrifuges will be used in this class. In all cases be certain that appropriate tubes or bottles are used—containers not designed for centrifugation may shatter or collapse under the forces generated in centrifugation. **Be certain that tubes and rotors are balanced.** In larger centrifuges tubes should be weighed and balanced relative to each other. Do not try to stop rotors manually. Be sure rotors are cleaned appropriately if spills have occurred.

4. **Gloves**:

Gloves must be worn when using hazardous chemicals. Some individuals develop allergies upon repeated exposure to certain types of gloves. This can result from either the material used or powders

applied to the glove. Inform me if you develop an allergic reaction or know that you are allergic to gloves—we will get you another type.

V. Chemical Hazards

1. MSDS (Material Safety Data Sheets) sheets are available in the lab for each chemical we use.

2. **Ethidium Bromide**:

Ethidium bromide (EtBr) staining is the most rapid and sensitive way of visualizing DNA in an agarose gel. EtBr is a planar molecule that complexes with DNA by intercalating into the double helix. When excited with UV light, EtBr fluoresces, allowing detection of DNA.

EtBr is a mutagen since it interferes with DNA replication when inserted into the double helix. Because it is a mutagen it also is a potential carcinogen and must be treated with respect.

EtBr solutions can be handled safely as long as **gloves** are worn. **All staining of gels will be done in a designated area in the prep room, and all stained gels and waste will be disposed of in labeled containers**. The staining solutions will be reused to minimize the generation of hazardous waste.

To further minimize risk, ethidium bromide solutions will be used at 1 µg/ml which is near the limit of detection of a significant effect in mutagenicity assays. Any solution or container containing EtBr will be labeled. Always wear gloves and safety glasses or goggles when handling solutions or stained gels.

3. **Other chemicals**:

You will be alerted to any other potential irritants, toxins, or teratogens at the start of any lab in which they are used, and necessary safety precautions will be described.